UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/586,720	07/20/2006	Claude V. Maina	NEB-238-PUS	4742

28986 7590 04/30/2009

HARRIET M. STRIMPEL, D. Phil.
New England Biolabs, Inc.
240 COUNTY ROAD
IPSWICH, MA 01938-2723

EXAMINER
GIBBS, TERRA C

ART UNIT	PAPER NUMBER
1635	

NOTIFICATION DATE	DELIVERY MODE
04/30/2009	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

STRIMPEL@NEB.COM
Goldberg@neb.com
wermuth@neb.com

Office Action Summary	Application No.	Applicant(s)	
	10/586,720	MAINA ET AL.	
	Examiner	Art Unit	
	TERRA C. GIBBS	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 January 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6 and 8-30* is/are pending in the application.

4a) Of the above claim(s) *12-30* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3, 5, 6, 8, 9, and 11* is/are rejected.

7)☒ Claim(s) *4 and 10* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This Office Action is a response to Applicant's Amendment and Remarks filed January 22, 2009.

Claim 7 has been canceled.

Claims 1, 5, 6, 8, and 9 have been amended.

Claims 1-6 and 8-30 are pending in the instant application.

This application contains claims 12-30 and mutations E38T, E38W, or E65A as recited in claim 4 and mutant E65A as recited in claim 10 drawn to an invention nonelected with traverse in the reply filed on May 13, 2008. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Accordingly, claims 1-6 and 8-11 have been examined on the merits.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Nucleotide Sequence Disclosures

In the previous Office Action mailed July 24, 2008, it was noted that this application failed to comply with the requirements of 37 C.F.R. §1.821-1.825. In view of Applicant's Amendment to the specification filed January 22, 2009, the instant application fully complies with the requirements of 37 C.F.R. §1.821-1.825.

Claim Rejections - 35 USC § 112

In the previous Office Action mailed July 24, 2008, claims 1-11 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. **This rejection is moot** against claim 7 in view of Applicant's Amendment filed January 22, 2009 to cancel this claim. **This rejection is withdrawn** against claims 5, 6, 8, 9, and 10 in view of Applicant's Amendment filed January 22, 2009 to remove the term, "hsiRNA" from the claims. **This rejection is maintained** against claims 1-4 and 11 for the reasons of record set forth in the previous Office Action mailed July 24, 2008.

Response to Arguments

In response to this rejection, Applicants contend that the claims have been amended accordingly to rely on the definition of "hsiRNA" as defined in the specification.

Applicant's contention has been fully considered, but is not found persuasive because claim 1 recites the term, "hsiRNA", where it appears that the specification has defined "hsiRNA" to be a heterogeneous short interfering double stranded RNA. Applicants is reminded that since abbreviations often have more than one meaning, it is suggested that inserting the full name of the hsiRNA would overcome the instant rejection.

Claim Rejections - 35 USC § 102

In the previous Office Action mailed July 24, 2008, claims 1-3 were rejected under 35 U.S.C. 102(b) as being anticipated by Blaszczyk et al. (Structure, 2001 Vol. 9, Issue 12, pages 1225-1236, reference #CB on Applicant's Information Disclosure Statement filed July 20, 2006). **This rejection is withdrawn** in view of Applicant's Amendment filed January 22, 2009. Specifically, the Examiner is withdrawing this rejection in view of Applicant's Amendment to claim 1 to recite that at least 15% of the fragments are not substantially degraded in the presence of mutant RNase III for at least one hour. It is noted that Blaszczyk et al. do not teach that the dsRNA/RNase III cleavage reaction was carried out for at least one hour.

In the previous Office Action mailed July 24, 2008, claims 1-3, 5-7 and 11 were rejected under 35 U.S.C. 102(b) as being anticipated by Sun et al. (Biochemistry, 2001 Vol. 40:14976-14984). **This rejection is moot** against claim 7 in view of Applicant's Amendment filed January 22, 2009 to cancel this claim. **This rejection is withdrawn** against claims 1-3, 5, 6, and 11 in view of Applicant's Amendment filed January 22, 2009. Specifically, the Examiner is withdrawing this rejection in view of Applicant's Amendment to claim 1 to recite that at least 15% of the fragments are not substantially degraded in the presence of mutant RNase III for at least one hour. It is noted that Sun et al. do not teach that the dsRNA/RNase III cleavage reaction was carried out for at least one hour.

Claim Rejections - 35 USC § 103

In the previous Office Action mailed July 24, 2008, claims 5, 8, and 9 were rejected under 35 U.S.C. 103(a) as being unpatentable over Sun et al. (Biochemistry, 2001 Vol. 40:14976-14984). **This rejection is withdrawn** in view of the new 35 USC § 103 rejection below. It is noted that Applicant's Amendment filed January 22, 2009 necessitated the new 35 USC § 103 rejection below.

Applicant's Amendment filed January 22, 2009 necessitated the new grounds of rejections presented below:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 5, 6, 8, 9, and 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Blaszczyk et al. (Structure, 2001 Vol. 9, Issue 12, pages 1225-1236, reference #CB on Applicant's Information Disclosure Statement filed July 20, 2006, of record), in view of Sun et al. (Biochemistry, 2001 Vol. 40:14976-14984, of record).

Claim 1 has been amended and is currently drawn to a method comprising reacting a preparation of large double-stranded RNA (dsRNA) with an effective amount of a mutant RNAse III to produce a heterogeneous mixture of fragments in which at least 15% of the fragments have a size of 18-25 nucleotides, wherein the at least 15% of the fragments are not substantially degraded in the presence of the effective amount of the mutant RNase III for at least 1 hour, the heterogeneous mixture being suitable for silencing gene expression. Claims 2 and 3 are dependent on claim 1 and include all the limitations of claim 1 with the further limitations wherein the mutant RNase III is contained in a magnesium or manganese buffer and wherein the mutant RNase III has a mutation in the position corresponding to E38 in *E. Coli* RNase III. Claim 5 has been amended and is currently drawn to a method comprising forming a heterogeneous mixture of fragments by incubating a large double-stranded RNA (dsRNA) with a mutant RNase III for an effective time for cleaving in the presence of magnesium ions or manganese ions, at least 90% of the large dsRNA as determined by gel electrophoresis and ethidium bromide staining wherein at least 30% of the cleaved dsRNA has a

fragment size of 18-30nt. Claims 6, 8, 9, and 11 are dependent on claim 5 and include all the limitations of claim 5 with the further limitations wherein the effective time period is about 1 minute to 20 hours; wherein the effective time is about 1 minute to 5 hours; wherein the effective time is about 1 minute to 10 hours; and wherein the large dsRNA has a length of at least 50 nt.

It is noted that Applicant's specification does not define what is an "effective amount" of a mutant RNase III enzyme. Therefore, the Examiner has interpreted the term, "effective amount" to be any amount of mutant RNase III enzyme that causes cleavage of dsRNA.

Determining the scope and contents of the prior art

Blaszczyk et al. disclose an *Aquifex aeolicus* RNase III/Mg^{2+} dsRNA model in which hydrolysis events cleave dsRNA substrates. Blaszczyk et al. teach that *E. Coli* RNase III proteins consist of an endonuclease domain and a double stranded RNA binding domain (dsRBD) (see Figure 1b). Blaszczyk et al. report point mutations causing defects in the dsRBD domain (see Figure 1b). Specifically, Blaszczyk et al. teach:

> "The crystal structure of the *A. aeolicus* endonuclease domain reveals that the polar acidic side chain of E37 forms a strong hydrogen bond with the amide group of E64 (Figure 4a), which is functionally essential, as suggested by the defective site-directed mutant E38V of Ec-RNase III (E37 in Aa-RNase III), which cannot form this hydrogen bond. In contrast, a glutamine variation at this position may not destroy the E37/E64-cutting site, because it can form a hydrogen bond with the amide group of E64 (E65 in Ec-RNase III). Our site-directed E → Q mutation at this position of Ec-RNase III indeed causes no genetic defect in RNase III function."

Indeed Blaszczyk et al. teach a substitution at amino acid E38, thus generating the E38V and the E38Q mutants (see page 1234, second column at Functional Analysis of the *rnc* Mutants).

Sun et al. teach the double-stranded-RNA processing activity of a truncated version of *E. Coli* RNase III lacking the dsRNA-binding domain. Sun et al. teach that a truncated form of Escherichia coli RNase III lacking the dsRBD RNase III domain can accurately cleave small processing substrates *in vitro*. Specifically, Sun et al. teach that *E. Coli* RNase III lacking the dsRNA-binding domain, in the presence of either Mg^{2+} or Mn^{2+} and for 25 or 30 minutes cleaved a 60 nt transcript dsRNA (see Figures 2 and 3, for example). Sun et al. also teach that the *E. Coli* RNase III lacking the dsRNA-binding domain cleaved at least 90% of its substrate (see Figure 4, for example). Sun et al. also teach that following cleavage by *E. Coli* RNase III lacking the dsRNA-binding, reaction products were electrophoresed and visualized by phosphoimaging in which primary and secondary (1° + 2°) products were observed (see Figures 2 and 3, for example). It is noted that the 1° + 2° products are approximately 25 nucleotides in length.

Ascertaining the differences between the prior art and the claims at issue.

Neither Blaszczyk et al. nor Sun et al. teach at least 15% of the fragments are not substantially degraded in the presence of mutant RNase III for at least one hour.

It is noted that Sun et al. taught a reaction time of 30 minutes and according to general knowledge and understanding in the art, based upon standard enzyme kinetics,

one of ordinary skill in the art would expect that the more time an enzyme is exposed to it's substrate, the more product would be produced.

Resolving the level of ordinary skill in the pertinent art

The level of ordinary skill in the pertinent art is considered to be high, being a graduate student or post-doctoral fellow in a biological science.

Considering objective evidence present in the application indicating obviousness or nonobviousness

It would have been *prima facie* obvious to one of ordinary skill in the art, at the time the invention was made to devise a method comprising reacting a preparation of large double-stranded RNA (dsRNA) with an effective amount of a mutant RNAse III to produce a heterogeneous mixture of fragments in which at least 15% of the fragments have a size of 18-25 nucleotides, wherein the at least 15% of the fragments are not substantially degraded in the presence of the effective amount of the mutant RNase III for at least 1 hour, the heterogeneous mixture being suitable for silencing gene expression using the teachings of Blaszczyk et al. in view of Sun et al., combined with general knowledge in the pertinent art of biological science. It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to devise a method comprising forming a heterogeneous mixture of fragments by incubating a large dsRNA with a mutant RNase III for an effective time for cleaving, in the presence of magnesium ions or manganese ions, at least 90% of the large dsRNA as determined by gel electrophoresis and ethidium bromide staining wherein at least 30% of the cleaved dsRNA has a fragment size of 18-30nt using the teachings of Blaszczyk et al. combined with Sun et al.

One of ordinary skill in the art would have been motivated to devise a method comprising reacting a preparation of large double-stranded RNA (dsRNA) with an effective amount of a mutant RNAse III to produce a heterogeneous mixture of fragments in which at least 15% of the fragments have a size of 18-25 nucleotides, wherein the at least 15% of the fragments are not substantially degraded in the presence of the effective amount of the mutant RNase III, the heterogeneous mixture being suitable for silencing gene expression since Sun et al. taught that such a method sheds light on the structure and function of *E. Coli* RNase III, an important enzyme which plays a key role in diverse prokaryotic and eukaryotic RNA maturation and degradation pathways. One of ordinary skill in the art would have been motivated to have the reaction time consist of at least 1 hour, for example, since Sun et al. taught a reaction time of 30 minutes and based upon standard enzyme kinetics, one of ordinary skill in the art would expect that the more time an enzyme is exposed to it's substrate, the more product would be produced.

One of ordinary skill in the art would have been motivated to have the RNase III mutant be a substitution at position E38 since Blaszczyk et al. taught that such a mutation causes no effect in RNase III function.

One of ordinary skill in the art would have had a reasonable expectation of success of devising a method comprising reacting a preparation of large double-stranded RNA (dsRNA) with an effective amount of a mutant RNAse III to produce a heterogeneous mixture of fragments in which at least 15% of the fragments have a size of 18-25 nucleotides, wherein the at least 15% of the fragments are not substantially

degraded in the presence of the effective amount of the mutant RNase III, the heterogeneous mixture being suitable for silencing gene expression since Sun et al. taught the successful use and design of such a method to elucidate the structure and function of *E. Coli* RNase III. One of ordinary skill in the art would have had a reasonable expectation of success of having the reaction time consist of at least 1 hour, for example, since using general knowledge known in the art at the time of filing, it was well known that the more time an enzyme is exposed to it's substrate, the more product would be produced. Therefore, one of ordinary skill in the pertinent art would have reasonably expected to have been able to achieve the instantly recited steps in one hour, for example, in view of that general knowledge.

Based on the teachings of Sun et al. and Blaszczyk et al., a person of ordinary skill has good reason to pursue claims 1-3, 5, 6, 8, 9, and 11 within his or her technical grasp. Since this leads to anticipated success, it is likely the product not of innovation, but of ordinary skill and common sense. Therefore, the invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of filing.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1 and 5 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is indefinite because the claim recites, "the heterogeneous mixture being suitable for silencing gene expression (hsiRNA)". It is unclear how the phrase, "the heterogeneous mixture being suitable for silencing gene expression" is being abbreviated as "hsiRNA" in claim 1 since it is clear that Applicant's specification has referred to "hsiRNA" to be a heterogeneous short interfering double stranded RNA molecule. Correction is required.

Claim 5 recites:

> A method comprising forming a heterogeneous mixture of fragments by incubating a large double-stranded RNA (dsRNA) with a mutant RNase III for an effective time for cleaving, in the presence of magnesium ions or manganese ions, at least 90% of the large dsRNA as determined by gel electrophoresis and ethidium bromide staining wherein at least 30% of the cleaved dsRNA has a fragment size of 18-30nt.

Claim 5 is indefinite because the claim is grammatically incorrect since the limitation, "at least 90% of the large dsRNA as determined by gel electrophoresis and ethidium bromide staining" appears to be incomplete.

Conclusion

Claims 4 and 10 are objected to as being dependent upon a rejected base claims, but would be allowable if rewritten in independent form to include all of the limitations of the base claim and any intervening claims. Claim 4 is considered to be free of the prior art since the prior art does not teach or fairly a method comprising reacting a preparation of large double-stranded RNA (dsRNA) with an effective amount of a mutant RNAse III to produce a heterogeneous mixture of fragments in which at least 15% of the fragments have a size of 18-25 nucleotides, wherein the at least 15%

of the fragments are not substantially degraded in the presence of the effective amount of the mutant RNase III for at least 1 hour, the heterogeneous mixture being suitable for silencing gene expression, wherein the mutant RNase III is E38A. Claim 10 is considered to be free of the prior art since the prior art does not teach or fairly a method comprising forming a heterogeneous mixture of fragments by incubating a large double-stranded RNA (dsRNA) with a mutant RNase III for an effective time for cleaving, in the presence of magnesium ions or manganese ions, at least 90% of the large dsRNA as determined by gel electrophoresis and ethidium bromide staining wherein at least 30% of the cleaved dsRNA has a fragment size of 18-30nt, wherein the mutant RNase III is E38A.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Terra C. Gibbs whose telephone number is 571-272-0758. The examiner can normally be reached from 9 am - 5 pm M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James “Doug” Schultz can be reached on 571-272-0763. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

April 24, 2009
/Terra Cotta Gibbs/

/Sean R McGarry/
Primary Examiner, Art Unit 1635